

Evaluation of substrates for mass multiplication of fungal biocontrol agents *Trichoderma harzianum* and *T. virens*

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Abstract

Evaluation of four substrates (neem cake, farm yard manure, coffee husk and tea waste) for the mass multiplication of fungal biocontrol agents *Trichoderma harzianum* and *T. virens* indicated that tea waste was the best media. The cultures mass multiplied in tea waste could also be stored for 3 months without much reduction in the population of biocontrol agents.

Key words : biocontrol agents, mass multiplication, *Trichoderma harzianum*, *T. virens*.

Diseases caused by soil-borne plant pathogens are serious production constraints in spice crops. Foot rot disease of black pepper and capsule rot (*azhukal*) of cardamom caused by *Phytophthora* spp. and rhizome rot of ginger caused by *Pythium* spp. cause heavy losses during monsoon seasons (Sarma *et al.* 1994). Although chemical control of these soil-borne pathogens reduces the disease to some extent; it is not cost-effective and eco-friendly. Biological control of soil-borne pathogens offers environmentally safe, durable and cost-effective alternative to chemicals. Fungal biocontrol agents such as *Trichoderma harzianum* and *T. virens* are effective in suppressing diseases in black pepper, cardamom and ginger (Bhai *et al.* 1993, 1994; Anandaraj & Sarma 1994; Sarma 1994). *T. harzianum* was also reported to be

a very effective biocontrol agent against *Sclerotium rolfsii* and *Rhizoctonia solani* (Hardar *et al.* 1979; Elad *et al.* 1983). Since these biocontrol agents are present in low populations in native soils, further augmentation of their density to reach a higher stability through artificial application is necessary. Earlier reports indicate the use of various agricultural wastes and by-products for multiplication of biocontrol agents (Kousalya & Jeyarajan 1990; Sawant & Sawant 1990; Bhai *et al.* 1994; Anandaraj & Sarma 1997). In this study we have evaluated some of the locally available substrates for their suitability for mass multiplication of fungal biocontrol agents.

One isolate each of *T. harzianum* and *T. virens* from the collection available at

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Indian Institute of Spices Research, Calicut was used in this study. The inoculum was prepared by growing *T. harzianum* and *T. virens* in petri plates containing Potato Dextrose Agar medium (PDA) and incubated at room temperature (28-39°C) for 3 days. From this, a 3-mm disc was cut using a cork borer and inoculated to 50 ml PDA medium in a 250 ml conical flask and incubated at room temperature for 4 days. From this culture, a spore suspension was prepared using sterile distilled water. For preparing the spore suspension, 50-ml sterile distilled water was poured into the conical flask and the mycelial mat scraped out aseptically using an inoculation loop and filtered into a sterilized conical flask through a cheese cloth. This procedure was repeated thrice to extract maximum inoculum concentration. The volume of the spore suspension was made up to 200 ml. The CFU was calculated at 10^5 dilution by plating in *Trichoderma* selective medium (Elad & Chet 1983). The CFU of *T. harzianum* and *T. virens* spore suspension were found to be 660×10^5 and 421×10^5 CFU/ml, respectively.

The substrates were moistened with water, neem cake and farm yard manure (FYM) (1:0.5 wt/vol), coffee husk (1:1 wt/vol), and tea waste (1:2.5 wt/vol), respectively; 200 g of these substrates were filled in 8" x 12" size polypropylene bags and tied with a rubber band. These bags were then sterilized in an autoclave at 121°C, 15 lbs/sq inch for 1 h. Two ml of the spore suspension was inoculated aseptically to each bag using a sterile syringe and shaken well to distribute the spores evenly to the substrates. The inoculated bags were kept in shelves at room temperature

(28-30°C). These bags were mixed well at 3 day intervals to distribute the growth evenly. To monitor the population of biocontrol agents, 1 g of each substrate was added in 9 ml sterile distilled water in a screw cap tube, and was diluted up to 10^5 level in the case of *T. virens* and 10^6 level in the case of *T. harzianum*. One ml from each dilution was plated in *Trichoderma* selective medium and incubated at room temperature for 6 days. Three replications were maintained for each dilution. Colonies were counted after 6 days of incubation. This was continued at 7 day intervals up to 4 weeks and at fortnightly intervals up to 3 months except for FYM, since it could not be stored for more than 1 month due to contamination and drying up of the substrate. Analysis of variance was carried out and the means were separated using Duncan's Multiple Range Test.

After 1 week of incubation, there was a significant increase in the population of *T. harzianum* in tea waste (192.3×10^6 CFU/g of the substrate). After 14 and 21 days, the population of *T. harzianum* in tea waste increased to 307×10^6 and 1780×10^6 CFU/g, respectively. The population of *T. harzianum* in coffee husk was 389×10^6 CFU/g after 21 days. The other two substrates showed less population when compared to tea waste and coffee husk. The population of *T. harzianum* in tea waste was 97×10^6 CFU/g of the substrate after 3 months of incubation. In the case of coffee husk, the population of *T. harzianum* was 27×10^6 CFU/g of the substrate. The population of *T. harzianum* was very less in neem cake (0.66×10^6 CFU/g of the substrate) (Table 1).

As in the case of *T. harzianum*,

Table 1. Population of *Trichoderma harzianum* on different substrates (CFU x 10⁶ dilution)

Treatment	Number of days after incubation							
	7	14	21	28	45	60	75	90
Coffee husk	4.00 ^b	14.00 ^c	92.33 ^c	125.33 ^b	91.00 ^b	67.33 ^b	50.33 ^b	25.00 ^b
Neem cake	2.33 ^b	11.30 ^c	63.00 ^c	40.66 ^c	23.33 ^c	15.66 ^c	9.66 ^c	7.33 ^c
Tea waste	293.30 ^a	490.00 ^a	502.33 ^a	829.33 ^a	792.00 ^a	555.33 ^a	380.00 ^a	277.33 ^a
Farm yard manure	1.00 ^c	65.33 ^b	202.66 ^b	156.66 ^b	-	-	-	-

- = Samples not taken

Figures followed by same letter within a column are non-significantly different at 0.05% level in Duncan's Multiple Range Test

maximum population of *T. virens* was found in tea waste 28 days after inoculation (829 x 10⁵ CFU/g of the substrate), followed by coffee husk (125 x 10⁵ CFU/g of the substrate). In the case of neem cake and FYM, maximum population of *T. virens* was noticed after 21 days of incubation and subsequently

in tea waste was 277 x 10⁵ CFU/g of the substrate. In coffee husk, the population was 25 x 10⁵ CFU/g of the substrate. As in the case of *T. harzianum*, the population of *T. virens* in neem cake was very less after 3 months (Table 2).

Table 2. Population of *Trichoderma virens* on different substrates (CFU x 10⁵ dilution)

Treatment	Number of days after incubation							
	7	14	21	28	45	60	75	90
Coffee husk	4.66 ^b	14.00 ^c	92.33 ^c	125.33 ^b	91.00 ^b	67.33 ^b	50.33 ^b	25.00 ^b
Neem cake	2.33 ^b	11.30 ^c	63.00 ^c	40.66 ^c	23.33 ^c	15.66 ^c	9.66 ^c	7.33 ^c
Tea waste	293.30 ^a	490.00 ^a	502.33 ^a	829.33 ^a	792.00 ^a	555.33 ^a	380.00 ^a	277.33 ^a
Farm yard manure	1.00 ^c	65.33 ^b	202.66 ^b	156.66 ^b	-	-	-	-

Samples not taken

Figures followed by same letter within a column are non-significantly different at 0.05% level in Duncan's Multiple Range Test

there was a slight reduction. In the case of tea waste and coffee husk, there was a reduction in population of *T. virens* after 28 days of incubation. The shelf life of cultures mass multiplied in tea waste was good when compared to the other substrates. After 90 days of incubation, the population of *T. virens*

From these observations it was concluded that tea waste support the best growth of both *T. harzianum* and *T. virens*. When compared to the population of *T. harzianum*, the population of *T. virens* in all the the four substrates was less. The cultures mass multiplied in tea waste and coffee husk can be

stored for 3 months without much reduction in the population of fungal biocontrol agents. This method is very simple and can be used for large-scale multiplication of fungal biocontrol agents.

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